

PATENT

Attorney Docket No.: A-72186
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

QI *et al.*

Serial No. 10/804,762

Filing Date: March 19, 2004

For: *Specific Inhibition of Allorejection*

Examiner: KELLY, Robert M.

Art Unit: 1633

**DECLARATION
Pursuant to § 1.132**

The undersigned, Dr. Uwe Staerz, hereby declares as follows:

1. I received my Ph.D. in Immunology in 1986. A copy of my curriculum vitae is attached. I am employed by Isogenis, Inc., the assignee of the above-referenced application and currently serve Chief Scientific Officer of the company.

2. I have read and am familiar with the disclosure in the above-referenced application and have reviewed as well the Examiner's comments in his most recent office action mailed May 2, 2006. I understand that the Examiner is concerned about the level of CD8 alpha chain expression in the allograft tissues and how it correlates with the immune inhibitory effect. As described in more detail herein, our own data in a solid organ transplant model demonstrates that only a fraction of the allograft cells actually need express the CD8 alpha chain in order for the immune inhibitory effect to be achieved *in vivo*.


3. In the experiment described in the patent specification at Example 3, standard pancreatic islet purification protocols were used. Adenoviral veto vectors had been produced as Adenoviral (Type 5) vectors. They were replication-deficient due to a lack of the E1 region (DE1). Genes coded within the E3 region had also been deleted to avoid the down-regulation of the MHC class I molecules on infected cells. The Adenoviral veto vector mAdCD8 carried the mouse CD8 alphachain as transgene, whose expression was controlled by a CMV immediate early promotor/enhancer.

4. Transduction conditions were optimized for the Adenoviral veto vector mAdCD8. An 18-hr transduction at the relatively low overall MOI of 3/1 resulted in efficient transduction and high viabilities of the small solid pancreatic islets. A control vector of similar composition carried beta-galactosidase as transgene. Immunohistology studies revealed that under these conditions approximately 50% of pancreatic islets showed detectable levels of the CD8 alphachain (Figure 1). In single cell suspensions approximately 18% of islet cells scored positive for surface expression (FACS, EPICS Coulter). The immunofluorescence staining of viable pancreatic islets also demonstrated that expression of the CD8 alphachain was especially found on cells of the surface layer of the pancreatic islets. The outside layer of cells efficiently expressed the veto molecule thus providing a barrier layer of CD8 alphachain expressing cells.

5. As described in the subject application, in control experiments mock-infected C57Bl/6 pancreatic islets were transplanted under the kidney capsule of streptozotocin-treated Balb/c mice. As seen in Figure 2, these islets were rejected. Another group of Balb/c recipients received C57Bl/6 pancreatic islets that had been transduced with mAdCD8. These animals received no additional immune suppressive therapy. mAdCD8-infected pancreatic islets were no longer rejected (Figure 2). After 45 weeks in the recipient animal, the overall histology of transduced allogeneic pancreatic islets was similar to that of islets transplanted into syngeneic hosts (Figure 3). Expression of the CD8 alphachain could be detected in some pancreatic islets.

6. Accordingly, as our data demonstrates, expression of CD8 alpha chain by every transplanted allograft cell is not required in order for the subject therapy to work *in vivo*, even with a solid organ transplant. Efficient transfection of the outside layer of allograft cells in the solid organ is sufficient to interact with responding T cells and inhibit their activity.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Uwe D. Stacz

04-29-06

Date

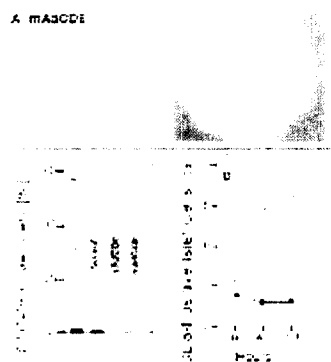


Figure 2: Purified pancreatic islets were transduced with mAdCD8 (A, red in D) or AdLacZ (B, black in D) for 12-hrs, incubated for an additional 24-hrs and evaluated immunohistologically for the expression of CD8. A peroxidase-labeled anti-CD8 antibody was used in (A) and a FITC-labeled one in (B), (C) and (D).

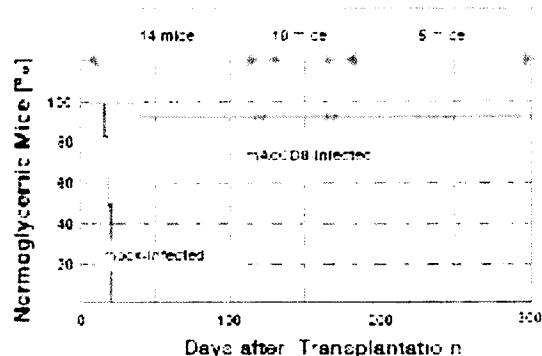


Figure 2 Survival of mock-infected and mAdCD8-transduced C57Bl/6 pancreatic islets in Balb/c mice.

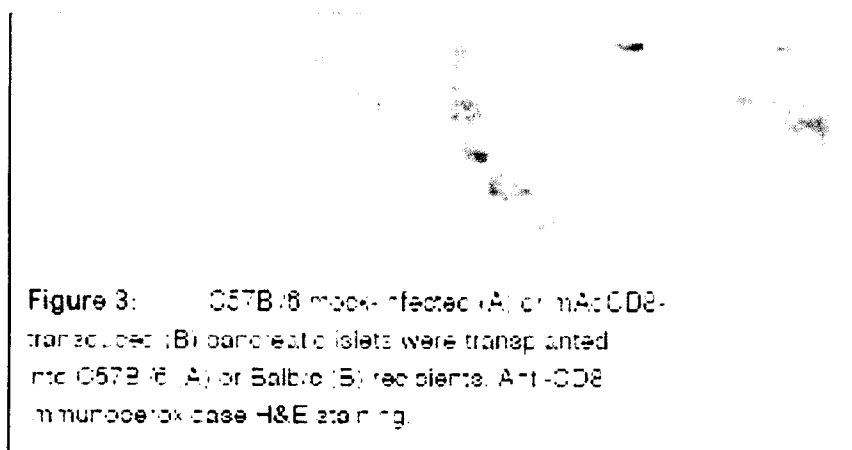


Figure 3: C57Bl/6 mock-infected (A) or mAdCD8-transduced (B) pancreatic islets were transplanted into C57Bl/6 (A) or Balb/c (B) recipients. Anti-CD8 immunoperoxidase H&E staining.

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EDUCATION

University Training:

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| 1977: | Eberhard-Karls-Universitaet, Tuebingen, Germany
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| 1979: | Eberhard-Karls-Universitaet, Tuebingen, Germany
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| 1982: | Eberhard-Karls-Universitaet, Tuebingen, Germany
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| 1986: | University of California, San Diego, CA, USA
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AWARDS

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| 1975-1982: | Studienstiftung des Deutschen Volkes |
| 1983: | Deutscher Akademischer Austauschdienst |

MEDICAL LICENSE

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| 1982: | Federal Republic of Germany |
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EXPERIENCE

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| 2000- | Chairman, Chief Scientific Officer, ISOGENIS, Inc., Denver
Colorado |
| 2004- | Senior Faculty Member, Institute for Therapeutic Biology, Denver,
Colorado |
| 2000 - 2003 | Senior Faculty Member, National Jewish Medical and Research
Center, Denver, Colorado |
| 2000 - 2005 | Professor, Department of Immunology, University of
Colorado Health Sciences Center, Denver, Colorado |

1995 - 2000	Associate Member, Department of Medicine, National Jewish Medical and Research Center, Denver, Colorado
1995 - 2000	Associate Professor, Department of Immunology, University of Colorado Health Sciences Center, Denver, Colorado
1990 - 2005	Member, Cancer Center, University of Colorado Health Sciences Center, Denver, Colorado
1994-1995	Interim Director of Animal Care Facility at the National Jewish Medical and Research Center, Denver, Colorado
1990-1994 Immunology, Denver, Colorado	Assistant Professor, Department of Microbiology and University of Colorado Health Sciences Center,
1989-1994	Assistant Member, Department of Medicine, National Jewish Medical and Research Center, Denver, CO, USA
1986-1989	Member, Basel Institute for Immunology, Basel, Switzerland
1983-1986	Research Fellow, Scripps Clinic and Research Foundation, La Jolla, California
1982:	Medical Resident in Clinical Pathology, Labor Dr. P. Schulz, Ludwigsburg, Germany

PROFESSIONAL MEMBERSHIPS

Member Review Committees NIH: Program Project on New Methods of Immune Intervention, on Thymic Involution, on Tuberculosis Diagnostics, on T Cell Development, on Vaccine Development and on Xenotransplantation

Member Review Committee Department of the Army: Prostate Cancer Treatment

Ad Hoc Member, Immunobiology Study Section, GDD Study Section

Ad Hoc Member, Immunology Review Committee - American Cancer Society

Associate Editor, Journal of Clinical Immunology

PATENTS

US Patent 5,078,998:

Hybrid Ligand Directed to Activation and Cytotoxic Effector T
Lymphocytes and Target Associated Antigen

US Patent 6,060,054 - Product for T lymphocyte
Immunosuppression;

US Patent 6,264,950 - Product and Process for T lymphocyte
Immunosuppression

PUBLICATIONS

1. Staerz, U.D., Pasternack, M.S., Klein, J.R., Benedetto, J.D. and Bevan, M.J. 1984. Monoclonal antibodies specific for a murine cytotoxic T-lymphocyte clone. **Proc. Natl. Acad. Sci. USA.** 81:1799.
2. Fink, P.J., Rammensee, H.-G., Benedetto, J.D., Staerz, U.D., Lefrancois, L. and Bevan, M.J. 1984. Studies on the mechanism of suppression of primary cytotoxic responses by cloned cytotoxic T lymphocytes. **J. Immunol.** 133:1769.
3. Staerz, U.D., Kanagawa, O. and Bevan, M.J. 1985. Hybrid antibodies can target sites for attack by T cells. **Nature.** 314:628.
4. Staerz, U.D., Rammensee, H.-G., Benedetto, J. and Bevan, M.J. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. **J. Immunol.** 134:3994.
5. Staerz, U.D. and Bevan, M.J. 1985. An allotypic determinant on the murine T cell receptor. In: **Molecular Biology of the Immune System.** J.W. Steilein, F. Ahmad, S. Black, B. Blomberg, and R.W. Voellmy, eds. Cambridge University Press, Cambridge, p. 61.
6. Crispe, I.N., Bevan, M.J. and Staerz, U.D. 1985. Selective activation of Lyt2+precursor T cells by ligation of the antigen receptor. **Nature.** 317:627.
7. Staerz, U.D. and Bevan, M.J. 1985. Cytotoxic T lymphocyte-mediated lysis via the Fc receptor of target cells. **Eur. J. Immunol.** 15:1172.
8. Staerz, U.D. and Bevan, M.J. 1986. Targeting cells for attack by cytotoxic T lymphocytes using heteroconjugates of monoclonal antibodies. **Symp. Fundam. Cancer Res.** 38: 31.
9. Staerz, U.D. and Bevan, M.J. 1986. Activation of resting T lymphocytes by a monoclonal antibody directed against an allotypic determinant on the T cell receptor. **Eur. J. Immunol.** 16:263.
10. Staerz, U.D. and Bevan, M.J. 1985. Use of heteroconjugates of antibodies to focus T cells to act at chosen sites. In: **Proceedings of UCLA Symposia**, Alan R. Liss, Inc., NY.
11. Staerz, U.D. and M.J. Bevan. 1986. Targeting cell attack by cytotoxic T lymphocytes using heteroconjugates of monoclonal antibodies. In: **Proceedings of the 38th Annual Symposium on Cancer Research. "Immunology and Cancer"**. The University of Texas Press, Houston, 38:81.
12. Staerz, U.D. and Bevan, M.J. 1986. The use of antibodies directed against the T cell receptor to focus the effector activities of T cells. In: **Mechanisms of Host Resistance to Infectious Agents, Tumors and Allografts.** The Rockefeller University Press, New York. p. 412.

13. Staerz, U.D. and Bevan, M.J. 1986. Hybrid hybridoma producing a bispecific monoclonal antibody which can focus effector T cell activity. **Proc. Natl. Acad. Sci. USA.** 83:1453.
14. Staerz, U.D., Lefrancois, L., and Bevan, M.J. 1986. Targeting for T lymphocyte-mediated lysis by hybrid antibodies. In: **Cytolytic Lymphocytes and Complement.** CRC Press, Inc., Boca Raton. p. 205.
15. Staerz, U.D. and Bevan, M.J. 1986. Use of anti-receptor antibodies to focus T cell activity. **Immunology Today.** 7:185.
16. Staerz, U.D., Yewdell, J.W. and Bevan, M.J. 1987. Hybrid antibody mediated lysis of virus infected cells. **Eur. J. Immunol.** 17:571.
17. Carbone, F.R., Staerz, U.D. and Paterson, Y. 1987. A new helper cell specificity within the pigeon cytochrome c determinant 95:104. **Eur. J. Immunol.** 17:897.
18. Lanzavecchia, A. and Staerz, U.D. 1987. Lysis of non-nucleated red blood cells by cytotoxic T lymphocytes. **Eur. J. Immunol.** 17:1073.
19. Staerz, U.D., Karasuyama, H. and Garner, A.E. 1987. Cytotoxic T lymphocytes against a soluble protein. **Nature.** 329:449.
20. Kappler, J.W., Staerz, U.D., White, J. and Marrack, P.C. 1988. T cell receptor Vb elements which recognize MLS-modified products of the major histocompatibility complex. **Nature.** 332:35.
21. Kisielow, P., Blüthmann, H., Staerz, U.D., Steinmetz, M. and von Boehmer, H. 1988. The mechanisms of self tolerance in T cell receptor transgenic mice. **Nature.** 333:742.
22. von Boehmer, H., Bluethmann, H., Staerz, U.D., Steinmetz, M. and Kisielow, P. 1988. Developmental biology of T lymphocytes. Deletion of autoreactive T cells and impact of the a, b-receptor on the CD4/CD8 phenotype. In: **Molecular Basis of the Immune Response**, C.A. Bona, ed.; Annals of the New York Academy of Sciences, 546:104.
23. Staerz, U.D. 1988. Targeting for lysis by CTL. In: **Human tumor antigens and specific tumor therapy**, UCLA Symposia on Molecular and Cellular Biology, New Series, R. Metzgar and M. Mitchell, eds.; Alan R. Liss, Inc., New York. 99: 209.
24. Zepp, F. and Staerz, U.D. 1988. Hybrid antibodies induce selection processes in the thymus. **Nature.** 336:474.
25. Staerz, U.D., Zepp, F., Schmid, R., Hill, M. and Rothbard, J. 1989. Recruitment of alloreactive cytotoxic T lymphocytes by an antigenic peptide. **Eur. J. Immunol.** 19:2191.

26. Staerz, U.D. and Bevan, M.J. 1989. Redirecting the Cytolytic Response. **International Reviews of Immunology**. 4:156.
27. Debrick, J.E., Campbell, P.A. and Staerz, U.D. 1991. Macrophages as accessory cells for class I MHC restricted immune responses. **J. Immunol.** 147:2846.
28. Chen, U., Kosco, M. and Staerz, U.D. 1992. Establishment and characterization of lymphoid and myeloid mixed-cell populations from mouse late embryoid bodies, "embryonic-stem-cell fetuses". **Proc. Natl. Acad. Sci. USA**. 89:2541.
29. Zepp, F. and Staerz, U.D. 1992. Monoklonale Hybrid-Antikoerper: Neue Perspektiven fuer Grundlagenforschung und Tumorthherapie. **Monatsschr Kinderheilkd.** 140: 745.
30. Kummer, U., and Staerz, U.D. 1993. Concepts of antibody-mediated cancer therapy. **Cancer Invest.** 11:147
31. Pawlowski, T., Elliott, J.D., Loh, D.Y., and Staerz, U.D. 1993. Positive selection of T lymphocytes on fibroblasts. **Nature**. 364: 642.
32. Pawlowski, P. and Staerz, UD. 1994. Positive selection in the thymus. **Behring Institute Res. Comm.** 94:94-103.
33. Candeias, S., and Staerz, U.D. 1995. T cell receptor V(-transcripts as early events of T lymphocyte development. **Eur. J. Immunol.** 24:3073.
34. Pawlowski, T. and Staerz, UD. 1995. Thymic education - T cells do it for themselves. **Immunology Today**. 15:205-209.
35. Carlsson, L., Candeas, S., Staerz, U.D., and Keller, G. 1995. Expression of FcγRIII defines distinct subpopulations of fetal liver B cell and myeloid precursors. **Eur. J. Immunol.** 25: 2308-2317.
36. Pawlowski, T. and Staerz, U.D. 1996. Thymic selection processes. **Intern. J. Immunopathology Pharmacology**. 2:150-162.
37. Pawlowski, T., Singleton, M., Loh, D.Y., and Staerz, U.D. 1996. Prosmiscuous recognition during positive selection. **Eur. J. Immunol.** 26:851-856.
38. Qi, Y., Singleton, M., Berg, R., and Staerz, U.D. 1996. T-cell veto induced by hybrid antibodies. **J. Exp. Med.** 183: 1873-1879.
39. Qi, Y. and Staerz, U.D. 1998. Specific Inhibition of CD4+ T lymphocyte by a Hybrid Antibody. **Nature Biotechnology** 16: 271-275 (with *Commentary Nature Biotechnology* 16: 237-238)
40. Princiotta, M., Bevan, M.J., Lenz, L., and Staerz, U.D. 1998. A bacterial attenuator as source of a T Lymphocyte antigen. **J. Exp. Med.** 187: 1711-1719

41. Konigsberg, P.J., Debrick, J.E., Pawlowski, T.J., and Staerz, U.D. 1999. Liposome encapsulated autothiomalate suppresses collagen-induced arthritis in DBA/1J mice. **Biochimica Biophysica Acta** 1421: 149-162
42. Berg, R. E., Moticka, J., Princiotta, M., and Staerz, U.D. 1999. Positive Selection of An H2-M3 Restricted T Cell Receptor. **Immunity**. 11: 33-43
43. Staerz, U.D., and Qi, Y. 1999. Treatment of An Autoimmune Disease with a Hybrid Antibody - A Proposal- **J. Clin. Immunol.** 19:195-202.
44. Staerz, U.D., and Qi, Y. 2000. Induction of Immune Unresponsiveness. **Immunology Today** 21: 172-176
45. Irion, S., Berg, R.E., and Staerz, U.D. 2000. A Physiological Ligand of Positive Selection is Recognized at High Specificity. **J. Immunol.** 164: 4601-4606
46. Berg, R.E., Irion, S., Princiotta, M.F., and Staerz, U.D. 2000. A Physiological Ligand of Positive Selection Induces T Cell Development at a Narrow Window of Avidity. **J. Immunol.** 165: 4209-4216
47. Kattman, S.P., Berg, R.E., Lukin, K., Oh, J., and Staerz, U.D. 2005. Maturation stage-dependent thymocyte responses to TCR engagement. **Eur J Immunol.** 35:2051-60.